

CHROMOSOMES IN A TRIPLOID GASTERIA.

(With Plate XI.)

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In most plants which have been studied in the genus *Gasteria* the haploid chromosome complement consists in four long and three short chromosomes. Three of the long chromosomes have subterminal centromeres and the fourth has its centromere farther removed from the end. The three short chromosomes have subterminal centromeres. This chromosome complement has been reported for eleven species and varieties by Ferguson (1926), for two species and some unidentified plants by Taylor (1924, 1931) and Tuan (1931), for twelve species by Marshak (1934), for fifteen species and varieties by Sato (1937), and for seventeen species and three unidentified plants by Resende (1937). In a preliminary report on the genus, the writer (Riley, 1945) has listed eleven species and varieties as well as five putative inter-specific hybrids as having the same complement.

A few plants of *Gasteria* with more than eight long and six short chromosomes have been recorded. Miss Ferguson has reported that one plant, *G. nigricans crassifolia*, was a probable tetraploid since it had twice as many long chromosomes as are found in most plants, but she was unable to obtain an exact count of the small chromosomes. Sato has reported a tetraploid plant of *G. maculata* which had 16 long and 12 short chromosomes in the somatic cells. Apparently no triploids have been found in *Gasteria* except one mentioned by the writer in his preliminary report, although Resende (1937) found three triploid plants of *Apicra bicarinata* which, he believes, were the only triploids found in the *Aloinae* up to that time.

Chromosome behaviour has been studied in a number of organisms that possess both large and small chromosomes. In some, chiasma frequency is proportional to chromosome length for all chromosomes and the small chromosomes sometimes fail to pair; in others the small chromosomes always pair. O'Mara (1931) found the latter situation in *Yucca flaccida*. This plant has five long and twenty-five short chromosomes. The long ones are 3μ long and have an average chiasma frequency

of three. The short chromosomes are only 0.4μ long but are all paired although a high percentage of univalents would be expected if chiasma frequency were strictly proportional to the length of the chromosomes. Figures illustrating a similar complete pairing of the small chromosomes of *Yucca filamentosa* and *Agave virginica* have been published by McKelvey and Sax (1933). Some other organisms in which pairing is not directly proportional have been listed by Darlington (1937). Apparently chiasma frequency is not directly proportional to length in organisms with localised pairing and in many that have general pairing if the short chromosomes are one-fourth the length of the long ones or are relatively shorter and if the short chromosomes are part of the normal chromosomal complement.

MATERIALS AND METHODS.

The plant discussed in this paper was one of a collection of succulents belonging to Professor Frank T. McFarland of the University of Kentucky. It was received in 1938 from the Huntington Botanical Gardens in California under the name *Gasteria sulcata* $\times$ *G. nigricans*.

Although this plant was labelled a hybrid, it more nearly resembles Baker's (1897) description of *G. nigricans*. The leafy stem is larger and the leaves more numerous than in *sulcata*. The leaves are generally shorter than those of *sulcata* and are dark green with a glossy surface like those of *nigricans*. The bracts also resemble those of *nigricans*. In the Flora Capensis the leaves of *sulcata* are described as being distichous or in a slightly twisted rosette and those of *nigricans* as distichous. The triploid resembles *sulcata* in this respect but is even more twisted (Plate XI). The leaves of the triploid appear to be thicker than those described in the Flora Capensis for either species or than the leaves on the specimens of *G. nigricans* or *G. sulcata* which we have available. This greater thickness, however, may merely be a reflection of the triploid nature of the plant. Berger (1908) describes the perigonium of *sulcata* as 25 mm. long and that of *nigricans* as 18 to 20 mm. The triploid was intermediate, having a perigonium 22 mm. in length.

All slides were prepared from fresh anthers by the original acetocarmine smear technique of Belling (1921). Examinations were carried out with a 2 mm., $90\times$ Bausch and Lomb apochromatic objective, N.A. 1.30, and a $15\times$ compensating ocular. Drawings were made with a camera lucida at a magnification of about $2850\times$ at drawing-board level and were subsequently reduced.

OBSERVATIONS.

Taylor (1931) has shown that the chromosomes of a diploid *Gasteria* line up on the metaphase plate of the first meiotic division with the three

pairs of small chromosomes at the centre of the figure and the four large bivalents radiating outward. At first anaphase one large bivalent usually separates slightly before the others.

TABLE 1.

The frequency of Microsporocytes with Varying Numbers of Quadrivalent (IV), Trivalent (III), Bivalent (II), and Univalent (I) Configurations in a Triploid Gasteria.

Large Chromosomes.					Small Chromosomes.				
IV.	III.	II.	I.	Freq.	III.	II.	I.	Freq.	
1	1	2	1	2	2	1	1	1	
0	4	0	0	93	1	2	2	3	
0	3	1	1	21	1	1	4	5	
0	2	3	0	1	1	0	6	3	
0	2	2	2	7	0	4	1	1	
0	1	3	3	1	0	3	3	23	
					0	2	5	37	
				125	0	1	7	30	
					0	0	9	22	
									125

To determine pairing relationships in the triploid, 125 cells in metaphase of the first meiotic division were studied. The various configurations that were observed are tabulated in Table 1. Because of their great difference in size the long and the short chromosomes are treated separately.

In 93 of the 125 cells, all twelve long chromosomes were associated in four trivalent configurations (Fig. 2). There were three trivalents with one bivalent and one univalent in 21 cells (Fig. 3). Seven cells had two trivalents, two bivalents, and two univalents, and one cell had one trivalent with three bivalents and three univalents. Three cells were more unusual. In one of these there were two trivalents but the six other long chromosomes were grouped as three bivalents. This arrangement suggests autosyndetic pairing between two chromosomes from one parent. In each of two other cells there was one trivalent, two bivalents, and one univalent, and the four other chromosomes were arranged in a quadrivalent configuration. A possible explanation for the quadrivalent is autosyndetic pairing between one of the chromosomes of a trivalent and a univalent chromosome. The 1,500 large chromosomes in the 125 cells were arranged in 2 quadrivalents, 454 trivalents, 45 bivalents, and 40 univalents.



FIGS. 2—7. Chromosomes during microsporogenesis in a triploid *Gasteria*. FIG. 2. Metaphase of the first meiotic division showing the long chromosomes arranged in four trivalents and the short ones in two bivalents, one bivalent and one univalent. FIG. 3. Very early anaphase of the first meiotic division; the long chromosomes are in three trivalents, one bivalent and one univalent and the short chromosomes in one bivalent and seven univalents. FIG. 4. First anaphase showing six long and three short chromosomes going to one pole, six long and six short to the other pole, a chromatid bridge and an accompanying fragment. FIGS. 5—7. Metaphase of the microspore division; in FIG. 5 there are 4 long and 5 short chromosomes, in FIG. 6, 5 long and 7 short, and in FIG. 7, 7 long, 4 short and one intermediate-sized chromosome. FIGS. 2 and 3, $\times 1050$; FIGS. 4, 6, and 7, $\times 1225$; FIG. 5, $\times 1350$ after reduction.

Apparently in the triploid the small chromosomes pair much less frequently than the large ones for there were relatively fewer trivalents and more univalents. Of the 125 cells, none had three trivalents, only one had two, and only eleven cells had one. On the other hand, 22 cells had nine univalents and 89 cells had five or more. The 1,125 short chromosomes were organised into 13 trivalents, 189 bivalents, and 708 univalents.

The third set of long chromosomes would be expected to be distributed at random at first anaphase. In 45 cells, the distribution was 4—8 in five, 5—7 in 19, and 6—6 in 42 cells. This is entirely in accord with expectation. The short chromosomes are more difficult to observe accurately at first anaphase as they are often completely or partially hidden by the long ones. In 33 cells the distribution appeared to be 4—5 and in 13 cells apparently 3—6. Four of the anaphase cells had chromatid bridges and in three of these a fragment was readily recognised (Fig. 4). Bridges and fragments were also found in a small percentage of the cells in first telophase.

Sax and Edmonds (1933) have shown that in sterile plants of *Tradescantia* the pollen begins to abort shortly after the end of the second meiotic division. Most of the microspores that will develop no further are small and have a large number of granules in the cytoplasm. In this triploid *Gasteria* 2,100 microspores were examined and about 22 per cent. were small. The nucleus appeared to be in a very early prophase and both the nucleus and cytoplasm were highly granulated and appeared to be disintegrating slowly.

Most of the microspores that were not obviously aborting were in the resting stage, but many were dividing. Of 655 cells in metaphase, 22 had four long chromosomes (Fig. 5), 154 had five (Fig. 6), 285 had six, 164 had seven (Fig. 7), and 30 had eight. There is an excess of the six-chromosome type over expectation. The fact that no cell has fewer than four or more than eight is to be expected from the configurations at first metaphase. The short chromosomes were clear in only 650 cells. Eighty-nine cells had three, 250 had four (Fig. 7), 205 had five (Fig. 5), and 97 had six. The high percentage of univalents at first metaphase indicates that some cells might have fewer than three or more than six. Actually, one cell had no short chromosomes, 2 cells had two, and 6 cells had seven (Fig. 6). The small percentage of cells with fewer than three or more than six short chromosomes may possibly be accounted for by the early abortion of most of the cells with the more unstable numbers. One of the cells that had four short chromosomes had seven long ones and one of medium size (Fig. 7). The medium-sized chromosome may have arisen by reciprocal translocation or fragmentation.

Over 750 mature pollen grains were examined in aceto-carmin and 46 per cent. appeared large, deeply-stained, and viable; the others were shrunken and obviously aborted.

DISCUSSION.

This plant is of interest because it is the first triploid reported in the genus *Gasteria*. Its origin is unknown but there is some reason to believe that it is a hybrid between *G. sulcata* and *G. nigricans*. Presumably it arose from the union of a gamete which developed without reduction and a normal, haploid gamete. This explanation is plausible, for the writer has observed a very small percentage of large pollen grains which are probably diploid in a number of plants of *Gasteria*. In addition, in one plant of *G. sulcata* seven microspores out of 422 had eight long and five or six short chromosomes, and these microspores were much larger than those with the usual four long chromosomes.

The high frequency of trivalent configurations among the long chromosomes indicates such a close homology among the three sets of chromosomes that this plant appears to be essentially an autotriploid. The high percentage of univalents among the short chromosomes, however, is not easy to explain. In the diploid plants which the writer has examined, the short chromosomes usually pair almost completely. This was found to be true in single individuals of *G. sulcata*, *G. laetepunctata*, *G. obtusifolia*, and *G. planifolia* $\times$ *G. sulcata* which had been received from the Huntington Botanical Garden, in a specimen of *G. acinacifolia* from the United States Bureau of Plant Industry, and in *G. nigricans*, *G. nigricans* var. *fasciata*, *G. parviflora*, and *G. nitida* from the National Botanic Gardens of South Africa at Kirstenbosch. Only in two hybrids from the Huntington Botanical Gardens labelled *G. pulchra* $\times$ *G. planifolia* and *G. verrucosa* $\times$ *G. brevifolia* were any univalents observed and they were relatively very few. In interpreting the relatively low frequency of pairing amongst the short chromosomes in the triploid, one might assume that the short chromosomes have a very short locus of chiasma formation. Perhaps the presence of three homologues causes a very slight delay in pairing which inhibits chiasma formation. In the diploids there are only two homologues, there is no difficulty about pairing and there are few univalents.

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PLATE XI.—A triploid Gasteria.